IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of :

Murray D. BAILEY, et al.

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Group Art Unit: 1625

Examiner: Binta M. Robinson

Confirmation No: 2759

For: Hepatitis C Inhibitor Peptide Analogs

DECLARATION UNDER 37 C.F.R. ' 1.132

Commissioner for Patents
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Sir:

I, Dr. Montse Llinas-Brunet, being duly warned, declare that:

I am a citizen of Canada, residing in Dollard-des-Ormeaux, Quebec, Canada.

I received a Ph.D. in Organic Chemistry from the University of Alberta, Edmonton, Alberta, Canada in 1986 and I have 22 years of experience as an organic and medicinal chemist.

I am presently Director in Chemistry at Boehringer Ingelheim (Canada) Ltd. My area of expertise is the medicinal chemistry of anti-viral compounds.

I am a co-inventor of the invention described and claimed in the above-identified application.

The following experiments were conducted under my supervision in Canada:

A cell-based luciferase reporter HCV RNA replication assay was performed to evaluate the cell-based activity for representative compounds of the above-identified application.

Cell culture: Huh-7 cells with a stable subgenomic HCV replicon that encodes a modified luciferase reporter gene (expressed as a luciferase-FMDV2A-neomycin phosphotransferase fusion gene) were established as previously described (Lohman et al., 1999. Science **285**: 110-113; Vroljik et al., 2003 J.Virol Methods **110**:201-209), with the exception that replicon cells were selected with 0.25 mg/ml G418. The amount of luciferase expressed by selected cells directly correlates with the level of HCV replication. These cells, designated as MP-1 cells, were maintained in Dulbecco's Modified Earle Medium (DMEM) supplemented with 10% FBS and 0.25 mg/ml neomycin (standard medium). The cells were passaged by trypsinization and frozen in 90% FBS/10% DMSO. During the assay, DMEM medium supplemented with 10% FBS, containing 0.5% DMSO and lacking neomycin, was used (Assay medium). The day of the assay, MP-1 cells were trypsinized and diluted to 100 000 cells/ml in assay medium. 100 µL was distributed into each well of a black 96-well ViewPlate™ (Packard). The plate was then incubated at 37°C with 5% CO_2 for two hours.

Preparation of test compound: The test compound in 100% DMSO was first diluted in assay medium to a final DMSO concentration of 0.5%. The solution was sonicated for 15 min and filtered through a 0.22 µM Millipore Filter unit. Into column 3 of a Polypropylene Deep-Well Titer Plate, the appropriate volume was transferred into assay medium to obtain the starting concentration (2x) to be tested. In columns 2 and 4 to 12, 200 µL of assay medium (containing 0.5% DMSO) was added. Serial dilutions (1/2) were prepared by transferring 200 µL from

column 3 to column 4, then from column 4 to column 5, serially through to column 11. Columns 2 and 12 were the no inhibition controls.

Addition of test compound to cells: A volume of 100μL from each well of the compound dilution plate was transferred to a corresponding well of the Cell Plate (Two columns were used as the "No inhibition control"; ten [10] columns were used for the dose response). The cell culture plate was incubated at 37°C with 5% CO_2 for 72 hours.

Luciferase assay: Following the 72h incubation period, the medium was aspirated from the 96-well assay plate and a volume of 100 μL of 1X Glo Lysis Buffer (Promega) previously warmed to room temperature was added to each well. The plate was incubated at room temperature for 10 min with occasional shaking. A black tape was put at the bottom of the plate. 100 μL of Bright-Glo luciferase substrate (Promega) previously warmed to room temperature was added to each well followed by gentle mixing. The luminescence was determined on a Packard Topcount instrument using the Data Mode Luminescence (CPS) with a count delay of 1 min and a count time of 2 sec.

The luminescence determination (CPS) in each well of the culture plate was a measure of the amount of HCV RNA replication in the presence of various concentrations of inhibitor. The % inhibition was calculated with the following equation:

% inhibition = 100- [CPS (inhibitor) / CPS (control) x 100]

A non-linear curve fit with the Hill model was applied to the inhibition-concentration data, and the 50% effective concentration (EC_{50}) was calculated by the use of SAS software (Statistical Software; SAS Institute, Inc. Cary, N.C.).

The general assay method is also described in published patent application WO

2005/028501.

Representative examples of compounds tested were: 2001, 2004, 2005, 2010, 3001, 4002, 4004, 4005, 5001, 6003 and 6004. Each of the compounds is a compound within the scope of the claims of the above-mentioned application. These examples display diversity in the substituents at R^1, R^2, R^4 and R^6.

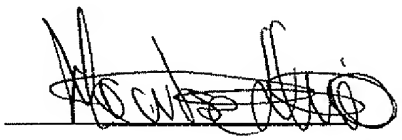
The results from the above-described cell-based luciferase reporter HCV RNA replication assay on these compounds were as follows:

Cpd	EC_{50} (nm)
2001	485
2004	255
2005	80.5
2010	445
3001	19.5
4002	28
4004	37
4005	85
5001	948
6003	305
6004	730

The data shows that the representative compounds, which have differing R^1, R^2, R^4 and/or R^6 substituents, exhibit cell-based activity in the cell-based luciferase reporter HCV RNA replication assay. This data also supports the statements in the application that the full scope of the compounds described can be used for the methods of treatment described in the application.

Based on the disclosure in the application, the supplemental data provided here and the knowledge already possessed by those in this technology field, one could use the invention in the manner described in the application, for example, to treat HCV infection.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.



Feb 21, 08

Dr. Montse Llinas-Brunet Date